43* Cascade carrier testing for cystic fibrosis: an Australian experience

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Introduction: In Victoria, Australia, cascade carrier testing is provided free of charge to people with a family history of cystic fibrosis (CF). Most children with CF are diagnosed by newborn screening which includes DNA testing. The uptake of cascade carrier testing following a child's diagnosis by newborn screening has not been previously described. The aim of this study is to report the uptake of cascade carrier testing by Victorian families.
Method: Examination of pedigrees of children with CF born 2001–2004 revealed the number of relatives eligible for cascade carrier testing. Relatives were eligible if they were aged 18 years or above and the family mutation was known. Uptake of cascade carrier testing was verified with the database of the DNA diagnostic laboratory (the only Victorian laboratory providing CF carrier testing).
Results: The pedigrees of 53 children were examined. Cascade carrier testing uptake was 16% (190/1160). Relationship to the child was associated with uptake; parents represented the greatest proportion of tested individuals (81/190) followed by aunts and uncles (47/190) and then grandparents (41/190). There was no difference in the uptake of cascade carrier testing between the maternal relatives and the paternal relatives. Four pedigrees indicated an existing family history of CF.
Discussion: The majority of relatives of children with CF have not had cascade carrier testing despite being at high risk. Investigation into barriers and facilitators to cascade carrier testing is being conducted to explore factors associated with uptake.

44* A French collaborative study indicative of a very low classical-CF penetrance of R117H; implications for genetic counselling

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Background: The R117H-associated phenotypes vary from classical CF to no clinical disease and have made genetic counselling difficult. Since implementation of CF NBS, the observed high R117H frequency among neonates with elevated IRT and two mutations has reinforced this issue.
Methods: Two retrospective studies were conducted: (1) a phenotypic study on 263 patients with two CFTR mutations including at least one R117H; (2) a retrospective 2002–2005 epidemiological study, aimed to determine the frequency of R117H and other frequent mutations in about 6000 healthy individuals without family history of CFTR pathology.
Results: (1) Among the 263 patients, including 92 neonates, detailed clinical features were available for 247: severe classical CF, n = 2; isolated CBAVD, n = 60; other CFTR-related disorders (CFTR-RD), n = 109; healthy, n = 76 (65 neonates, reduced follow-up period); (2) Based on R117H and F508del allelic frequencies in the general population of 0.25% and 1.0%, respectively, the [F508del]+[R117H] genotype prevalence was evaluated at 1/20,000, the CFTR-RD penetrance at 4.2% and the CF penetrance at 0.06%.
Conclusion: The very low penetrance of R117H with regard to classical CF leads to consider R117H no longer as a CF-causing mutation and to reassure patients and their families in view of genetic counselling.
Supported by: Vaincre la Mucoviscidose.

45 Prenatal genetic diagnosis in cystic fibrosis by ARMS-PCR method and STR genotyping

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Objectives: The aim of this study was to detect CFTR mutations in genomic DNA isolated from amniotic fluid collected by amniocentesis at couples with previous family history of cystic fibrosis and to verify the absence of contamination with maternal blood or cells by STR genotyping, in order to provide a correct genetic counseling.
Method and Materials: Based on family history or typical echographic findings, 8 couples were selected for prenatal diagnosis. Genomic DNA was isolated from amniotic fluid collected by transabdominal amniocentesis in the 16th week of pregnancy and maternal venous blood collected on EDTA. The genetic analysis for CFTR mutations was performed with Elucigene CF29 kit which uses the ARMS-PCR method. We performed STR genotyping for several loci by polyacrilamide gel electrophoresis in order to verify the absence of contamination with maternal blood or cells of the amniotic fluid.
Results: we identified 3 heterozygous genotypes (ΔF508/N, G542X/N), four normal genotypes and one compound heterozygote (621+1G>T/ΔF508). The STR genotyping results showed the absence of contamination with maternal DNA of fetal DNA.
Conclusions: Prenatal diagnosis can be performed by ARMS-PCR using Elucigene CF29 kit only in cases were the detected genotype has at least one allele with ΔF508 mutation or is a compound heterozygote for the other mutations detected by the kit. STR genotyping is a good complementary method which can be used for obtaining a correct prenatal diagnosis.
Supported by: Research Grant-CNCSIS A/1188/2004–2006.

46 Molecular strategy in hyperechogenic fetal bowel

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Hyperechogenic fetal bowel (HFB) is detected in 0.1–1.8% of pregnancies during the second or third trimester, and could constitutes a prenatal sign of CF. It has been reported that the prevalence of meconium ileus (MI), an early manifestation of CF, is higher among neonates with previous HFB than in neonates with normal ultrasound imaging. There could be a relationship between HFB and MI.
The aims were to evaluate usefulness and limits of genetic analysis in couples with HFB, and to define the best testing strategy based on a review of CF patients with MI.
Results of genetic analysis on 79 Italian CF patients with MI, and 47 HFB couples were reviewed.
Screening for the most frequent mutations in 79 MI patients allowed a 90.5% detection rate, which increased to 96.8% after DNA sequencing, and to 98.1% after MLPA; two couples of carriers were identified, and in both prenatal diagnosis confirmed CF in the fetus. A third couple, whose child carried F508del and P5L, chose to terminate the pregnancy. In a further pregnancy the fetus again had HFB, and prenatal diagnosis showed heterozygosity for F508del. Because of the severity of the ultrasound picture, this pregnancy was terminated.
Testing the most frequent mutations and deletions covers 91.8% of CFTR mutations in CF with MI. A similar testing strategy may be appropriate in HFB. We do not suggest to sequence the gene, because of the possibility of identifying mutations with unknown/unclear clinical consequences. We also suggest to proceed with extreme caution when counselling parents of a fetus with HFB who are carriers of mild or novel mutations, as their presence in the fetus does not justify a diagnosis of CF.